Concentration of Phytase in Alfalfa Juice by Ultrafiltration

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Introduction

Using transgenic alfalfa to produce enzymes such as phytase has many attractions including the potential to produce large quantities of enzyme at a lower cost than more typical microbiological fermentations (Austin and Bingham 1996). Phytase has an application as a monogastric feed supplement, to improve the animal's utilization of organic phosphate in the diet and therefore to reduce the environmental impact of the manure.

Product recovery from alfalfa typically involves extraction of juice from the fiber and subsequent processing of the juice to obtain the desired product. This can be a difficult downstream processing problem due to the complex composition of the extracted juice. The problem may be somewhat simplified in the case of phytase because the protein in the juice may be incorporated into the ration thus reducing the purification needs. Concentration of extracted juice in the field would also reduce transport costs to a processing plant. The aim of this study was to evaluate the potential of dynamic ultrafiltration to concentrate alfalfa juice containing phytase.

Materials and Methods

Ultrafiltration was carried out in a pilot apparatus (VSEP) (New Logic International, Emeryville, CA) fitted with a 10,000 molecular weight cutoff (MWC) regenerated cellulose membrane. The unit consisted of an annular membrane (area of 0.0465 m^2) that was vibrated by a torsion spring at 60 Hz creating high shear forces at the juice-membrane interface, thereby increasing mass transfer rates over traditional cross flow apparatus. The apparatus was operated with an amplitude of vibration of approximately 25.4 mm at the periphery, and a transmembrane pressure of 100 psi. Filtration was carried out without pretreatment and at temperatures between 15 and 35°C. Initial sample volume was approximately 40 l and retentate was recycled to achieve concentration.

Results and Discussion

Phytase activity. Phytase activity increased in the retentate fraction throughout the experiment but activity expressed per g DM was reduced after about 6h (Fig. 1). This may have been due to long processing times or loss of activity due to stress from mechanical shear with entrained air or from dehydration. A final DM concentration of 20 to 25% was achieved when the whole juice was processed.

There was some phytase activity in the permeate after 4h but this disappeared at later times (Fig. 1). The appearance of activity in the permeate indicates that phytase, which is typically reported to have a subunit MW of 40 to 60 kD, is not totally rejected by a membrane with a nominal MWC of 10 kD. It is often found that the nominal membrane MWC is not closely related to its rejection of a particular molecule, thus creating the requirement for extensive testing of membranes to determine particular process performance. The disappearance of activity from the permeate at later times may be due to fouling, resulting in a decrease in the effective MWC of the membrane

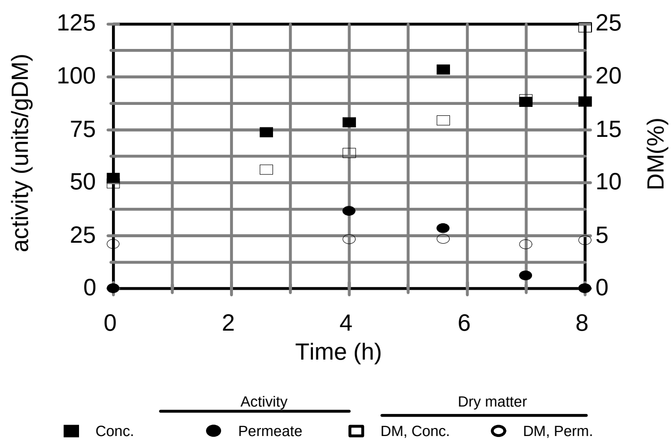

Figure 1. Phytase activity and juice DM during concentration of alfalfa juice by ultrafiltration through a 10 kD regenerated cellulose membrane. An activity of one unit is release of m-mole inorganic phosphorus per minute from excess phytate at 37°C.

and subsequent dilution of the phytase in the permeate fraction. The loss of activity over time is also likely to be a contributing factor.

Processing rates. The ability of a processing unit to concentrate the juice at a rate compatible with expected harvesting rates is a performance criterion of any proposed field apparatus. To satisfy typical field conditions, it was calculated that a permeation rate of 600 ml/m^{2}min was the minimum acceptable. This is the rate that would allow the juice collected over a 10 h harvesting period to be concentrated from 12 to 20% DM in a 20 h period. From the graph of permeation rate (Fig. 2), it appears that this minimum standard could be reached with the full scale VSEP unit (27.9 m^2 membrane area) although linear scaleability was assumed, which may not be correct.

The fouling index (FI) in Figure 2 was calculated as the flux of water before membrane use, divided by the measured permeate flux. The membrane had a lower initial water flux, and a much lower FI, than other tested membranes with greater nominal pore sizes. The ideal situation would be a high water flux and a low FI to produce high processing rates for the alfalfa juice.

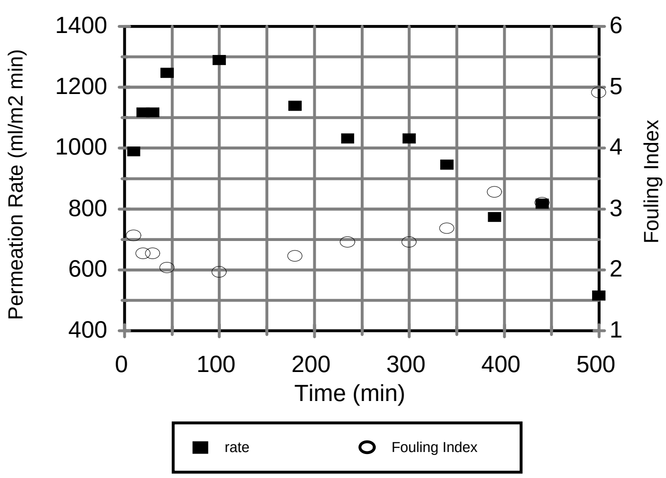

Figure 2. Permeation rate of alfalfa juice and fouling index on 10 kD regenerated cellulose ultrafiltration membrane at a transmembrane pressure of 100 psi.

There was some evidence that the permeation rate was affected by temperature which affects the viscosity of the feed. The concentration of the juice also affects viscosity and there appeared to be a limit of around 20% DM in the retentate stream for satisfactory permeation rates.

Conclusions

The performance of the VSEP was superior to previous attempts to concentrate whole alfalfa juice by ultrafiltration (Ostrowski-Meissner 1983). There appears to be considerable potential to use dynamic filtration to concentrate alfalfa juice in the field, although there are still many issues to be resolved. The phytase was concentrated in the retentate fraction which indicates that, at present, the only processing option is concentration of the juice. Attempts to fractionate the larger chloroplastic protein from the smaller soluble protein fractions, including the phytase, using larger membrane pore sizes were not successful because fouling reduced the effective MWC of the membranes. It is important to identify and characterize the performance of suitable membranes and operating conditions for processing alfalfa juice.

There are also several pretreatment options to remove the chloroplastic protein and allow better UF performance. These include temperature and pH precipitation which are well established in alfalfa juice processing.

References

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